

## ENHANCEMENT OF MECHANICAL PERFORMANCE BY STRETCH DURING TETANIC CONTRACTIONS OF VERTEBRATE SKELETAL MUSCLE FIBRES

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### SUMMARY

1. Single fibres from the semitendinosus muscle of *Rana temporaria* were stretched during fused tetanic contractions and tension and sarcomere length (laser diffraction) responses were recorded.

2. Stretch of the fibres caused proportional increases in length of the sarcomeres. The force increased to a plateau value which was maintained during stretch or increased slightly.

3. The plateau value of force during stretch was dependent upon the velocity of stretch, was independent of the amplitude of stretch and was not proportional to overlap of thick and thin filaments.

4. There was enhancement of force *after* stretch compared with that produced at the same sarcomere length during isometric tetani. This force enhancement was independent of the velocity at which the stretch had been applied.

5. At sarcomere lengths between 1.9 and 2.3  $\mu\text{m}$ , the force enhancement after stretch decayed rapidly, was independent of amplitude of stretch above approximately 25 nm per sarcomere and was not associated with a shift of the force-velocity curve. At sarcomere lengths above 2.3  $\mu\text{m}$  the force enhancement after stretch decayed very slowly and was still present after 4 sec in long tetani.

6. At sarcomere lengths above 2.3  $\mu\text{m}$ , force enhancement after stretch increased with amplitude of stretch and increased for any given stretch amplitude with sarcomere length. The force recorded after stretch was thus not proportional to overlap of thick and thin filaments.

7. At sarcomere lengths above 2.3  $\mu\text{m}$ , the force enhancement after stretch was associated with a shift towards higher force values of the force-velocity curve. The velocity of shortening at zero load ( $V_{\text{max}}$ ) derived by hyperbolic extrapolation of the force-velocity curve was not affected.

8. Tension enhancement during and after stretch has a stabilizing effect in preventing dispersion of sarcomere length, particularly on the descending limb of the length-tension curve.

\* The experiments were performed at this address.

## INTRODUCTION

The confirmation and detailed delineation of the length-tension curve of muscle in single fibres (Ramsey & Street, 1940; Gordon, Huxley & Julian, 1966; Edman, 1966) established that relationship as a fundamental basic description of muscle function. The fact that movement, i.e. length changes during contraction, alters the mechanical performance has been less fully appreciated. It has recently been confirmed in single fibres that shortening during contraction reduces mechanical performance (Edman, 1975).

Similarly the enhancement of force by stretch of actively contracting muscle has been known for many years in whole muscle (Fenn, 1924; Abbot & Aubert, 1951; Hill & Howarth, 1959). Deleze (1961) was struck by the fact that this force after the end of a stretch is maintained at a higher level even if the stretch takes the muscle to the descending limb of the length-tension curve. He pointed out the problem of explaining this increase of force with decreasing overlap of thick and thin filaments. More recently, Cavagna & Citterio (1974) showed that the enhancement by stretch involved an upward shift of the force-velocity curve since muscles stretched during activity were able to shorten at finite velocity at loads higher than  $P_0$ .

The purpose of the present study was to elucidate the force enhancement by stretch in greater detail in single fibres. Measurements have been carried out over a considerable part of the length-tension diagram and the mechanical behaviour correlated with changes in sarcomere length measured by laser diffraction techniques. Some of these results have been reported in brief previously (Edman, Elzinga & Noble, 1976).

## METHODS

**Preparation.** Twenty-four single fibres from the semitendinosus muscle of *Rana temporaria* were studied. The fibres were mounted in a similar manner to that previously described (Edman & Kiessling, 1971; Edman, 1975). A schematic drawing of the apparatus is shown in Text-fig. 1. The fibre A was mounted between a tension transducer C and a lever D connected to the moving coil of an electromagnetic puller E. The position of the tension transducer and the length of the fibre were adjusted by means of a micrometer screw N.

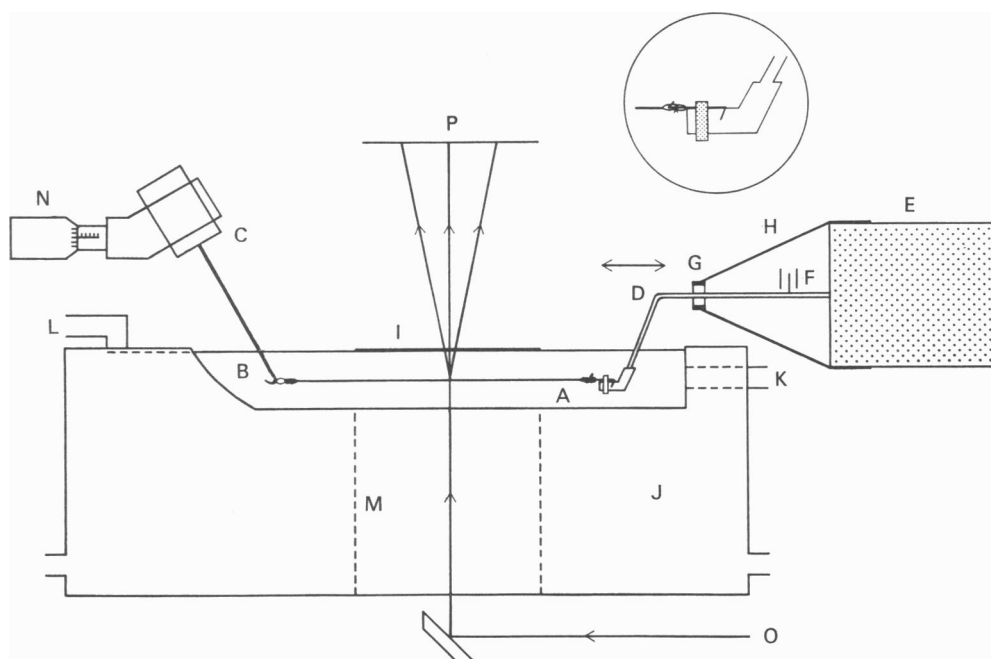
**Solution.** The composition of the bathing solution was (mM): NaCl 115.5, KCl 2.0,  $\text{CaCl}_2$  1.8, Na phosphate buffer 2.0, pH 7.0. The solution was exchanged before each series of paced contractions. The temperature was controlled by circulating a water-glycol solution through the jacket of the muscle chamber from a Colara Ultrathermostat. This maintained the temperature constant to  $\pm 0.2^\circ\text{C}$  during the experiment; the temperature varied from  $0.8$  to  $2.75^\circ\text{C}$  between different experiments.

**Stimulation.** Stimulus pulses were fed to two parallel platinum plates placed on either side of the fibre 2 mm from it. Rectangular pulses of 0.2 msec duration were used. The stimulus strength was approximately 25% above threshold. A 1 sec train of pulses (frequency 16–22 Hz) was given to produce a fused tetanus. In some experiments, described below, pulse trains of longer duration were applied. Each fibre was paced by inducing tetani regularly at 2 min intervals (except in the case of 5 sec tetani when 5 min intervals were used). The fibre was paced for at least 20 min before the actual experiment began. This type of pacing produced a stable contractile performance over many hours. Damage of fibres was detected by decreased speed of upstroke of tension in complete fusion, tension drop during the tetanus plateau and/or increased dispersion of sarcomere length.

**Tension recording.** An RCA 5734 mechano-electric transducer was used, with a glass attachment and hook for connecting the fibre. The glass attachment and hook compliance was  $1.5\ \mu\text{m}/$

mN. The frequency response was approximately 750 Hz with the lever immersed in the bath fluid. The resting tension was 2–8% of  $P_0$  at the highest length studied (2.85  $\mu\text{m}$ ). Stretch ramps similar to those applied during tetani were also applied to some resting fibres. The tensions so produced at times corresponding to the time of measurement of force enhancement after stretch were 1.7–2.8% of  $P_0$  at a sarcomere length of 2.85  $\mu\text{m}$ . The corresponding value *during* stretch was 2.0–5.1% of  $P_0$  depending on velocity of stretch. No correction was made for these contributions by resting tension in the determination of force enhancement by stretch during activity.

*Electromagnetic puller and servo system.* This was identical to that used previously (Edman, 1975). Load clamps (released to selected controlled force) were performed as described by Edman & Hwang (1977).



Text-fig. 1. A, muscle fibre. B, muscle fibre chamber. C, tension transducer. D, lever movable in the horizontal plane. E, electromagnetic vibrator. F, displacement transducer. G, Teflon ring. H, brackets. I, glass slide placed on top of Ringer solution. J, jacket for circulation of thermostatically controlled water-glycol mixture. K, inlet for bath solution. L, suction drain. M, air space for passage of laser beam. N, micrometer screw. O, direction of laser beam. P, screen for recording laser diffraction pattern. Inset: detail of end of lever D showing attachment of tendon.

*Sarcomere length recording.* The laser diffraction technique described by Cleworth & Edman (1972) was used for recording of sarcomere length in different segments along the length of the fibre. A helium-neon laser (Spectra-Physics model 124A, continuous wave) was used with a polaroid filter to control intensity. The light beam (1.5 mm diameter) was reflected up through the fibre chamber by a front surfaced mirror placed underneath; this mirror was mounted on a screw, allowing adjustment for study of any part of the length of the fibre. A glass cover-slip I (Fig. 1) of 0.1 mm thickness was placed on the surface of the bathing solution. The diffraction pattern was displayed on a partially transmitting horizontal screen P placed 150 mm above the fibre. For initial adjustment and direct visualization a screen with a grid calibrated in sarcomere length was used. For streak photography a second screen was placed in position. This screen was provided with a slit (1.5 mm width) placed across the diffraction pattern. Movements of the first order beam relative to the zero order reference were recorded on continuously moving film (Gevapan at 50 or 100 mm/sec with a Grass Oscilloscope camera). The sarcomere spacing was

calculated from the equation  $d = n\lambda/\sin \theta_n$ , where  $n$  is the order of the diffraction line,  $\lambda$  the wave-length of the light (632 nm),  $d$  the sarcomere spacing and  $\theta_n$  the angle between the  $n$ th and zero order lines (determined from the arc-tangent). On those occasions when streak photography was not carried out, changes in sarcomere length in response to various amounts of stretch during activity were calculated from the change in overall fibre length and the sarcomere length at the onset of the lever movement. This change in length was measured with a capacitance type displacement transducer used and calibrated as described by Edman (1975).

The intensity of the first order streak line was estimated from the original film records. The absorbance of monochromatic light (550 nm wave-length) by the first order streak line was measured using an M 85 Vickers scanning and integrating microdensitometer. Measurements made at intervals across the first order line, corresponding to 5 nm intervals of sarcomere length, yielded plots of relative intensity against displacement (Pl. 1D).

*Determination of fibre length and cross-sectional area.* The length between the insertions of the fibre to the tendons was measured to the nearest 0.5 mm with a Zeiss Stereo II microscope (6 $\times$  magnification) at the end of each experiment. The fibre was then mounted in a separate trough in which it could be turned 90° along its longitudinal axis. Two fibre diameters were measured at 500 $\times$  magnification with a Zeiss Standard Universal microscope at 1 mm intervals along the length of the fibre. Cross-sectional area was calculated at each point assuming an elliptical shape. The mean value was obtained from 8–10 such measurements.

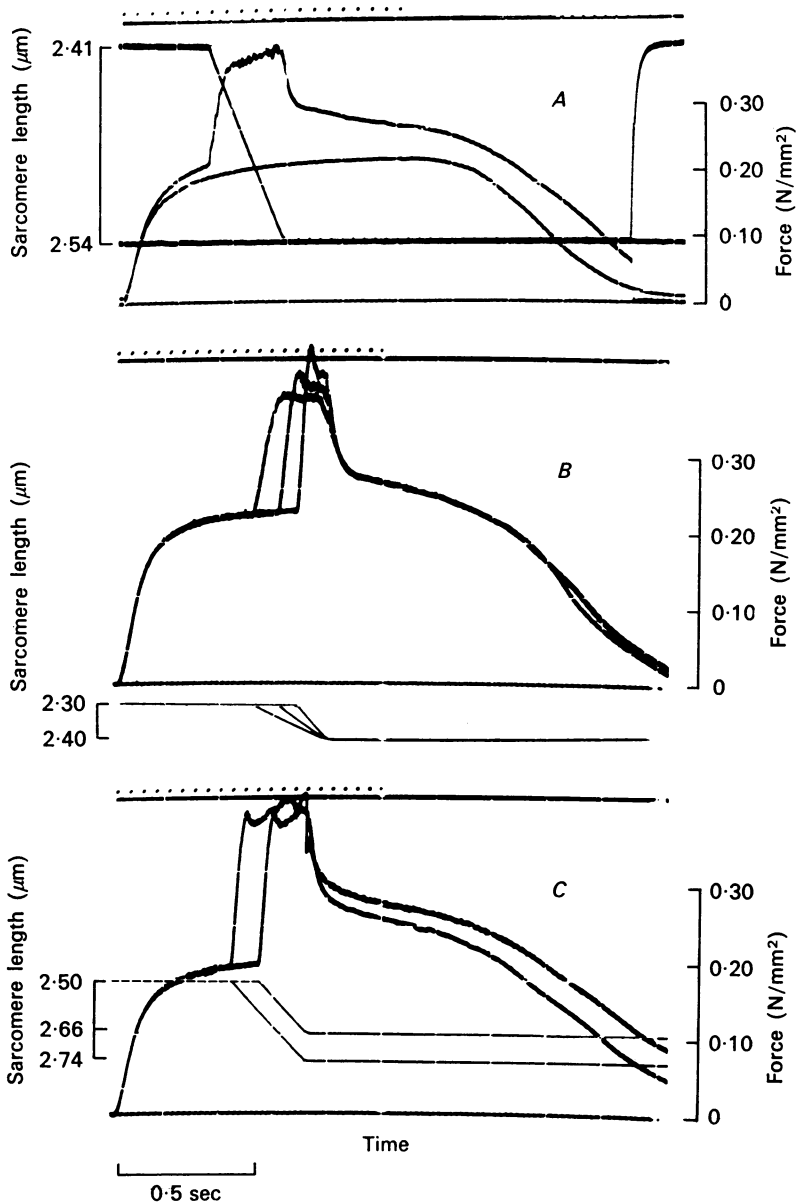
*Analysis.* Tension and length change were displayed on a Tetrax 5103N storage oscilloscope and photographed on 35 mm film with a Cossor camera. Measurements from the film (oscilloscope and laser diffraction recordings) were made on a Nikon comparator. Fitting of Hill's hyperbolic equation (Hill, 1938) to the force-velocity data by means of a computer program was carried out in the same manner as that described previously (Edman, Mulieri & Scubon-Mulieri, 1976). However, no truncation of data at loads above 0.8  $P_0$  was made.

## RESULTS

When fibres were stretched during contraction an increased force was recorded. As can be seen in Text-fig. 2A, tension rose rapidly at first, then it remained constant or increased slowly during the remainder of the stretching period. This phenomenon will be called 'force enhancement during stretch'. The force decayed at the end of the stretch but remained higher than the force recorded in a control isometric contraction at the long (stretched) length (Text-fig. 2A). This phenomenon will be called 'force enhancement after stretch'.

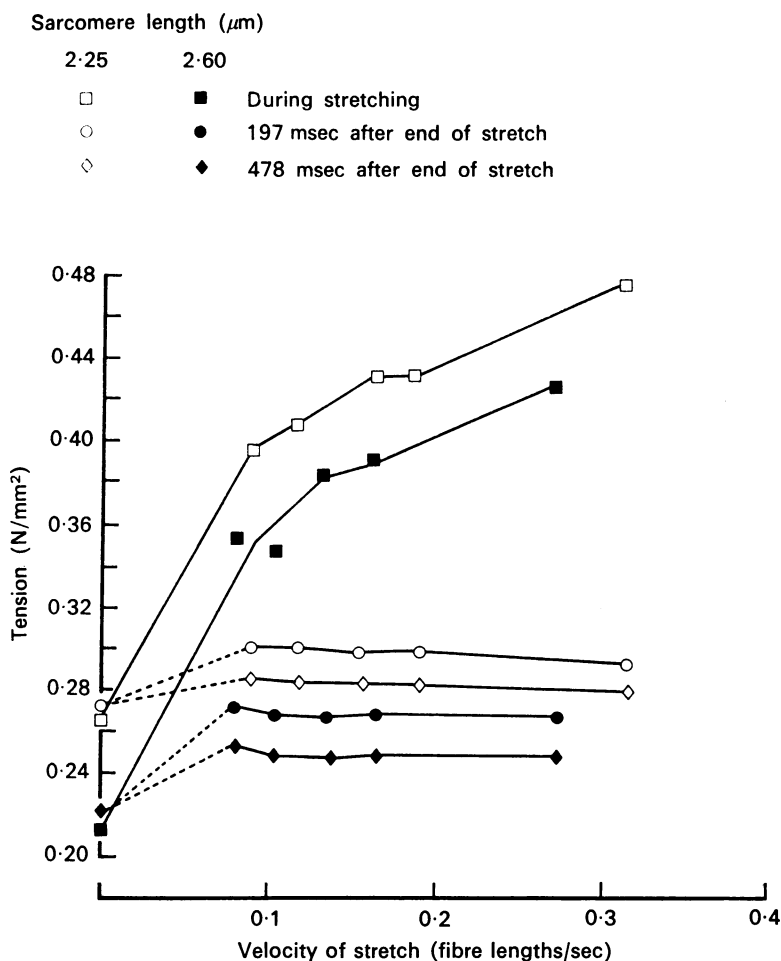
### *Force enhancement during stretch*

*Relationship to stretch velocity, stretch amplitude and sarcomere length.* The magnitude of the force recorded during stretch was dependent upon the velocity of stretch, i.e. the faster the fibre was stretched, the higher the recorded force (Text-figs. 2B and 3). This corresponds to the negative part of the force-velocity relationship. The *shape* of the force record was also dependent on the velocity of stretch (Text-fig. 2B). At low velocities, the initial rapid rise of tension was followed by a slow rise or flat plateau of force during stretch. At higher velocities on the other hand, force attained an initial peak after which it declined to a steady level or increased slowly during the remainder of the stretching phase (Text-fig. 2). There were sometimes small tension oscillations during this phase. The possibility that these were due to minute oscillations of the lever during the ramp could not be excluded; the amplitude of such oscillations would need to be of the order of 5  $\mu$ m in order to produce the largest force oscillations encountered (stiffness value from Cleworth & Edman, 1972). The magnitude of force enhancement for a given velocity was independent of amplitude of



Text-fig. 2. Force and displacement records from single fibres stretched during tetanus. Stimulus markers are indicated at the top of each record. *A*, comparison of stretch during activity from  $2.41 \mu\text{m}$  sarcomere length to  $2.54 \mu\text{m}$  with ordinary isometric tetanus at  $2.54 \mu\text{m}$  sarcomere length (base line slightly shifted upwards). *B*, comparison of three stretches during activity from  $2.30$  to  $2.40 \mu\text{m}$  sarcomere length; different velocities of stretch were performed so that the three stretches ended at the same time during the tetani. *C*, comparison of a stretch during activity from  $2.50$  to  $2.66 \mu\text{m}$  sarcomere length with one from  $2.50$  to  $2.74 \mu\text{m}$ ; the different amplitudes of stretch were performed at the same velocity and timed so that the two stretches ended at the same time during the tetani. Temperature,  $2.75^\circ\text{C}$ . Cross-sectional area: *A*,  $6.952 \times 10^{-3} \text{ mm}^2$ ; *B*, *C*,  $8.595 \times 10^{-3} \text{ mm}^2$ .

stretch; the longer stretches merely prolonged the constant or slowly rising second phase of force enhancement during stretch (Text-fig. 2C). When a velocity of stretch was chosen which gave a flat-topped shape of force record at  $1.95\text{--}2.25\ \mu\text{m}$  sarcomere length, an increase in sarcomere length to above  $2.3\ \mu\text{m}$  caused the force to increase slowly during the second phase of force enhancement during stretch. The relationship between force during stretch and sarcomere length is shown in Text-fig. 4A. It

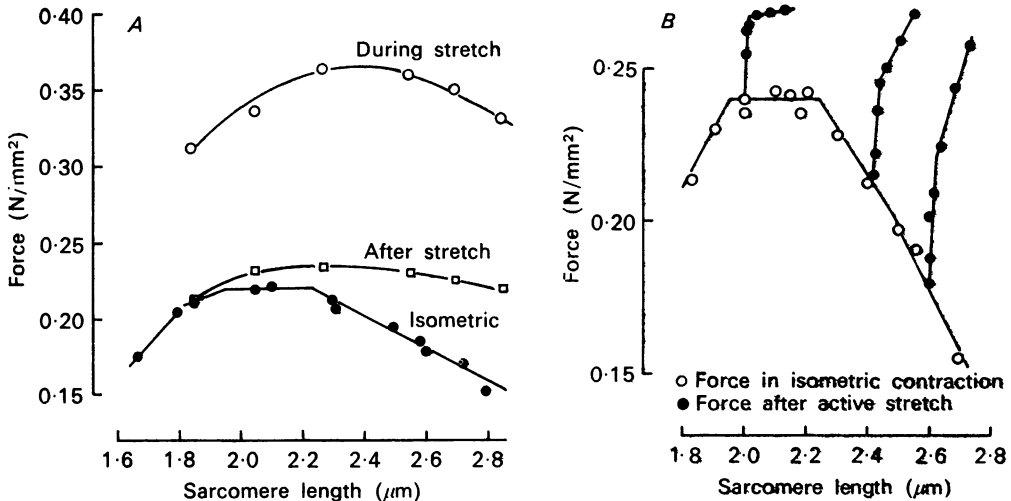


Text-fig. 3. Total force during and after stretch as a function of velocity of stretch at two different sarcomere lengths. The points on the ordinate indicate the force during the ordinary isometric tetani. Temperature:  $1.49^\circ\text{C}$ . Cross-sectional area:  $10.312 \times 10^{-3}\ \text{mm}^2$ . Fibre length (used for calculation of velocity):  $13.0\ \text{mm}$  at sarcomere length  $2.25\ \mu\text{m}$ ;  $15.0\ \text{mm}$  at sarcomere length  $2.60\ \mu\text{m}$ .

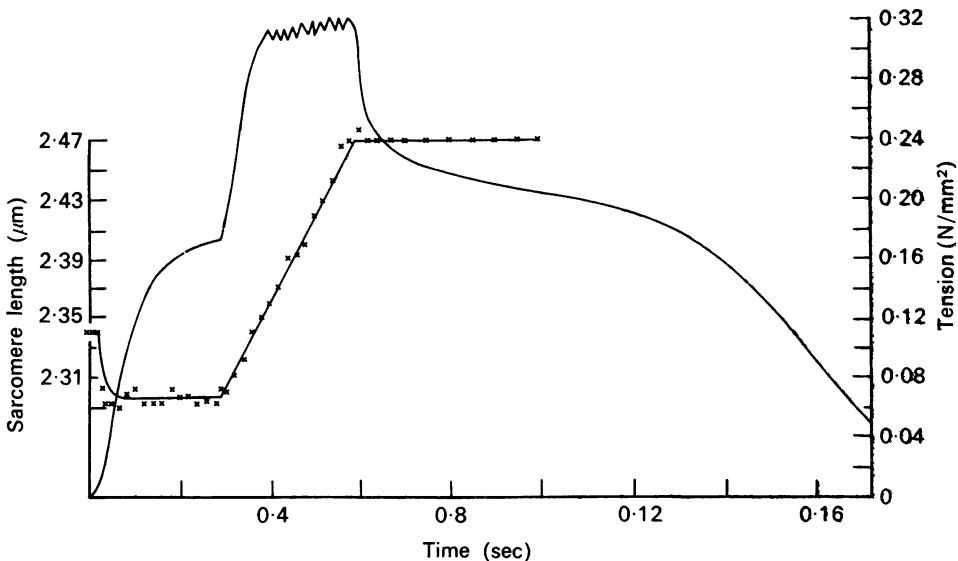
can be seen that the force during stretch was maximal at sarcomere lengths  $2.2\text{--}2.3\ \mu\text{m}$  and was not proportional to the isometric tension over the range of sarcomere lengths studied (Text-fig. 4A).

*Sarcomere length measurements.* A streak photograph of the light diffraction pattern is shown in Pl. 1. In accordance with previous observations (Cleworth & Edman,

1972), there was an outward movement of the first order beam (in relation to the zero order reference) during the rising phase of the isometric tetanus indicating shortening of the sarcomeres. During stretch the first order moved nearer the zero order beam reflecting a lengthening of the sarcomeres. There was a change in the substructures during stretch as is indicated by the enlarged portion of the streak



Text-fig. 4. Relationship between total force and sarcomere length. *A*, forces during and after stretches of constant velocity and amplitude ( $0.2 \mu\text{m}$ ) compared with the isometric values. *B*, effect of increasing amplitude of stretch at three different starting points on the isometric length-tension curve. Temperature: *A*,  $1.49^\circ\text{C}$ ; *B*,  $0.84^\circ\text{C}$ . Cross-sectional area: *A*,  $10.312 \times 10^{-3} \text{ mm}^2$ ; *B*,  $5.255 \times 10^{-3} \text{ mm}^2$ .



Text-fig. 5. Effect of stretch on tension and sarcomere length during a tetanus. Measurements of sarcomere length (crosses) made from a streak photograph are superimposed on traced tension record. Temperature,  $1.08^\circ\text{C}$ . Cross-sectional area:  $19.914 \times 10^{-3} \text{ mm}^2$ .

record (Pl. 1C). The fine streak lines were discontinuous in that some individual lines faded and others increased in intensity. It should be noted that the total width of the first order line remained very nearly constant during stretching, and that there was no fall of overall intensity or peak intensity (Pl. 1D). Similar records were obtained at 0.5 mm intervals along the length of an individual fibre in sequential tetani.

The changes in sarcomere length measured (at  $20\times$  magnification in a Nikon comparator) from the middle of the first order streak line relative to the zero order reference are plotted with the corresponding force record in Fig. 5. With the time resolution of recording used, and visual estimation of the central position of the first and zero order beams, it was not possible to detect any discontinuities in the sarcomere length changes during stretch as previously reported by Flitney & Hirst (1975) in studies of frog whole sartorius muscle. The straightness of the streak record during stretch is furthermore evident from Pl. 1C.

#### *Force enhancement after stretch*

*Time course of force enhancement after stretch.* In the experimental protocols described above, the force enhancement after stretch decayed with time. In order to determine the time course of the decay, 5 sec tetani were studied. Examples of these are shown in Text-fig. 6. It was found that at the plateau of the length-tension curve (e.g.  $2.07\ \mu\text{m}$  in Text-fig. 6A) the force enhancement died away completely at approximately 1 sec after the end of the stretch. By contrast at long sarcomere lengths (e.g.  $2.55\ \mu\text{m}$  in Text-fig. 6B), there was a phase of relatively rapid decay during the first sec after the end of the stretch, but this was followed by continued or maintained force above control. Considerable enhancement (4–5 %) remained at the end of the stimulation period 4.3 sec after the end of the stretch.

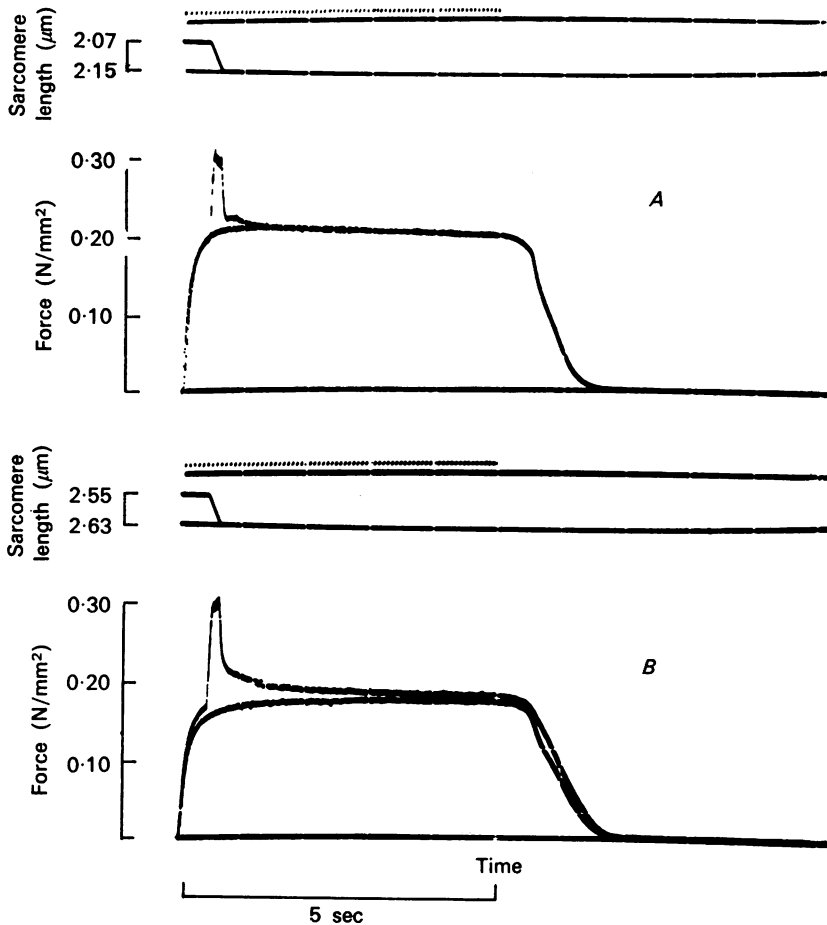
*Relationship of force enhancement after stretch to sarcomere length, stretch amplitude and velocity of preceding stretch.* Practical considerations dictated that the experiments be carried out using 1 sec tetani at 2 min intervals. Thus the force enhancement after stretch found at the plateau of the length-tension curve was still present, i.e. the force enhancement that was found to die away in the 5 sec tetani (above). Tension measurements were always made at the same time during contraction.

The magnitude of the extra force recorded after the end of the stretch was not dependent on the velocity at which the fibre had been stretched (Text-figs. 2B and 3). When stretches of the same amplitude but different velocities were arranged to end at the same time during the tetanus (Text-fig. 2B) there was an initial rapid fall of force from the different preceding peak values; the subsequent slower decay of force followed a common path. The measurements in the remainder of the description of results were made after the steep part of this decay, i.e. from 150 msec after the end of stretch.

For a given amplitude of stretch the force enhancement after stretch increased with sarcomere length over the range explored ( $1.8$ – $2.8\ \mu\text{m}$ ). In Text-fig. 4A the length-tension curve is plotted for control isometric tetani and compared with the enhanced force after stretch. As can be seen (Text-fig. 4A) there was no force enhancement at sarcomere lengths below  $1.8\ \mu\text{m}$ . The increasing enhancement with increasing sarcomere length resulted in a relationship between tension and sarcomere length after stretch quite different in shape and slope from that recorded in the control



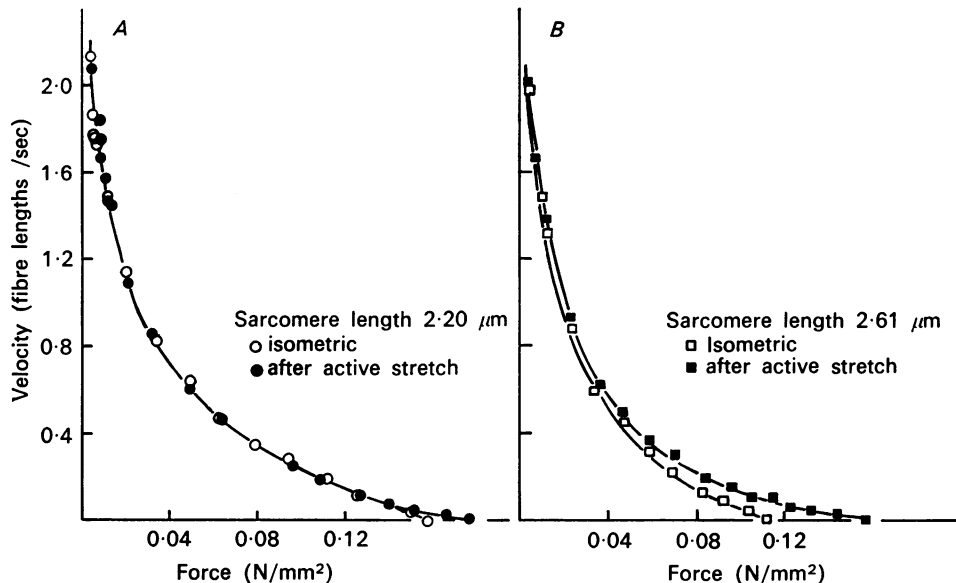
isometric series. It should be noted that the peak tension after stretch at approximately  $2.5\ \mu\text{m}$  sarcomere length was clearly higher than the ordinary isometric tension at the plateau of the length-tension curve at  $2.1\ \mu\text{m}$  sarcomere length (Text-fig. 4*A*).



Text-fig. 6. Force and displacement records from single fibre stretched during long 5 sec tetani. Stimulus markers are indicated at the top of each record. *A*, comparison of stretch during activity from  $2.07\ \mu\text{m}$  sarcomere length to  $2.15$  with ordinary isometric tetanus at  $2.15\ \mu\text{m}$  sarcomere length. *B*, comparison of stretch from  $2.55$  to  $2.63\ \mu\text{m}$  with isometric tetanus at  $2.63\ \mu\text{m}$ . Temperature,  $0.84\ ^\circ\text{C}$ . Cross-sectional area:  $5.255 \times 10^{-3}\ \text{mm}^2$ .

The relationship between total force and amplitude of sarcomere extension during activity was explored by varying the amplitude of stretch. Different curves were obtained, depending on the sarcomere length used as a starting point. Three such curves superimposed on the ordinary length-tension curve are illustrated in Text-fig. 4*B*. When contractions starting in the range of sarcomere length  $1.95$ – $2.25\ \mu\text{m}$  were studied, force enhancement was found to be virtually independent of amplitude over a wide range. Very small amplitude stretches (up to approximately  $25\ \text{nm}$  per sarcomere)

were required to produce maximum force enhancement at this sarcomere length; further increases in amplitude of stretch produced no or only minor further force enhancement. The resulting relationship between length and tension after active stretch therefore had a very steep positive initial slope, a sharp angle and quite a flat slope with little length dependence of force over the remainder of the range studied (Text-fig. 4*B*). When stretches were performed at sarcomere lengths above  $2.3\ \mu\text{m}$ , the tension continued to rise over the entire range of amplitudes studied so that the slope of the relationship between stretch amplitude and tension above the angle was definitely positive (Text-fig. 4*B*). This slope increased with further increase of initial



Text-fig. 7. Force-velocity relationships during ordinary isometric tetanus (open symbols) and after ( $0.14\ \mu\text{m/sarcomere}$ ) stretch during tetanus (filled symbols) at sarcomere lengths of  $2.20\ \mu\text{m}$  (left) and  $2.61\ \mu\text{m}$  (right). Velocity of shortening was obtained from the displacement record during shortening at constant load. Fibre length used for calculation was that at  $2.25\ \mu\text{m}$  sarcomere length. Temperature,  $3.0^\circ\text{C}$ . Cross-sectional area:  $7.676 \times 10^{-3}\ \text{mm}^2$ . Experiment of 14.6.76 (Table 1).

sarcomere length. Thus at initial sarcomere lengths above  $2.6\ \mu\text{m}$  a very steep positive relationship between length and tension after active stretch was found over the entire range (Text-fig. 4*B*). Curves of the type plotted in Text-fig. 4*A* were obtained by joining points of a given amplitude of stretch from each individual active length-tension curve plotted in Text-fig. 4*B*.

*Force-velocity relationship after stretch.* Force-velocity curves were recorded by releasing the fibres to isotonic loads after the end of the stretch and compared with similar curves derived from releases at the same time during control isometric contractions. There was a departure from a hyperbolic curve in the force-velocity relationships for loads above  $0.8 P_0$  at sarcomere lengths  $1.95$ – $2.27\ \mu\text{m}$  as previously observed (Edman, Mulieri & Scubon-Mulieri, 1976). This departure was reduced in the curves obtained after active stretch (Text-fig. 7), stretch caused a higher isometric

force ( $P_0$ ) and slightly higher velocities of shortening at isotonic loads above 94 % of  $P_0$ . However, all velocities recorded at loads lower than this were indistinguishable, i.e. the force-velocity curves with and without prior active stretch were virtually identical except at the very highest loads (Text-fig. 7).

At sarcomere lengths above  $2.3 \mu\text{m}$  there was a more distinct separation over the entire range of loads of the force-velocity curves derived after stretch and without preceding stretch, and this difference increased in magnitude with increasing load

TABLE 1. Force and velocity of shortening with and without preceding stretch during tetanic contractions at different sarcomere lengths. C = control; S = after stretch.  $l$  = fibre length at  $2.25 \mu\text{m}$  sarcomere length.

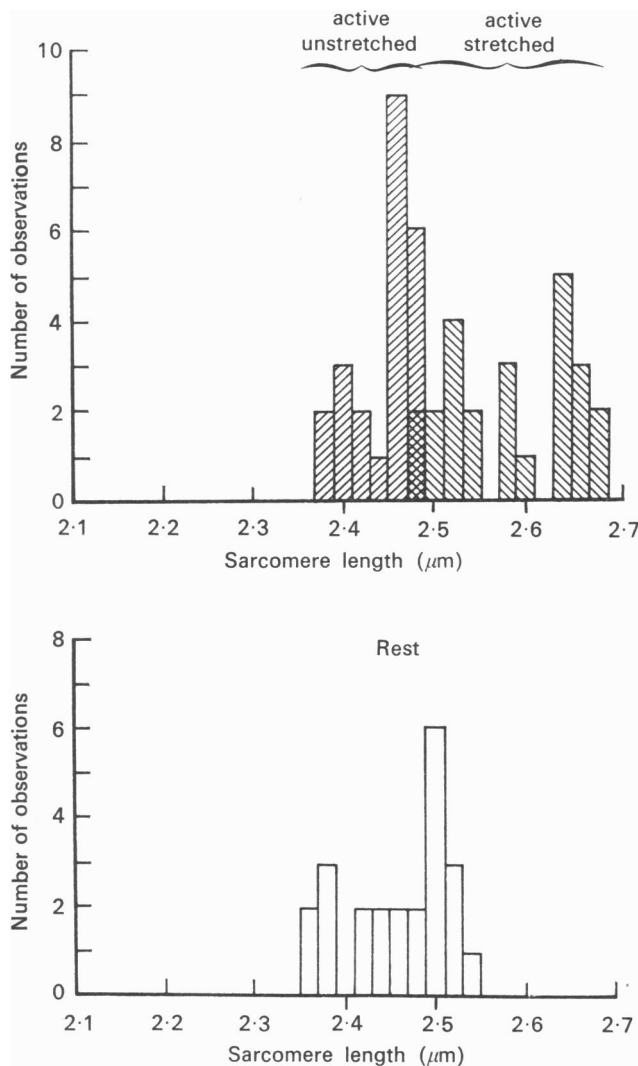
Date of experiment	Sarcomere length ( $\mu\text{m}$ )	$V_{\text{max}}$ (l/sec) (computed)	$P_0$ (N.mm $^{-2}$ ) (measured)	Velocity at 50% control $P_0$ (l/sec) (measured)
14.10.76	2.57 C	1.87	0.227	0.195
	2.57 S	1.65	0.314	0.220
21.10.76	2.42 C	1.39	0.230	0.175
	2.42 S	1.44	0.272	0.205
22.10.76	2.49 C	1.99	0.204	0.305
	2.49 S	2.01	0.247	0.340
10.6.76	2.15 C	2.18	0.201	0.405
	2.15 S	2.32	0.231	0.405
11.6.76	2.27 C	2.39	0.167	0.325
	2.27 S	2.46	0.192	0.325
	2.56 C	2.18	0.132	0.315
	2.56 S	2.19	0.162	0.340
14.6.76	2.20 C	2.51	0.158	0.360
	2.20 S	2.60	0.177	0.360
	2.61 C	2.51	0.111	0.395
	2.61 S	2.50	0.157	0.445

(Text-fig. 7, Table 1). Force-velocity data were fitted to Hill's equation by a computer program and  $V_{\text{max}}$  was calculated. These results are shown in Table 1, together with the measured values for  $P_0$  and the velocity at 50 % of  $P_0$ . For sarcomere lengths up to  $2.27 \mu\text{m}$  there were no differences between the values with and without stretch for  $V_{\text{max}}$  and the velocity of shortening at 50 %  $P_0$  (three pairs of values). At sarcomere lengths  $2.42$  and above, five pairs of data were available, allowing calculation of the probability of a difference between stretch and control being due to chance. This was done with the Sign test with a correction for continuity (Snedecor & Cochran, 1967). According to this analysis velocity of shortening at 50 %  $P_0$  increased ( $P = 0.05$ ) and the velocity at zero load ( $V_{\text{max}}$ ) was not changed ( $P > 0.05$ ).

*Sarcomere length measurements.* After the end of the stretch, the position and internal substructure of the first order beam remained extremely stable until the beginning of relaxation (Text-fig. 5, Pl. 1).

Frequency histograms of sarcomere length recorded in one single fibre at different points along its length are presented in Text-fig. 8. In fibres which maintained consistent mechanical performance over hours and showed no sign of damage, there was

no great difference in variance of sarcomere length during rest, isometric contraction and after stretch. Dispersion of sarcomere length after stretch did occur in fibres which developed damage, but in this case there was also increased dispersion and non-uniform shortening of sarcomeres during ordinary isometric contractions.



Text-fig. 8. Frequency histograms of sarcomere lengths obtained from measurements made along the whole length of the fibre. Above: distribution during plateau of the ordinary isometric tetanus and after stretch during tetanus. Below: distribution of sarcomere lengths of the unstimulated fibre. Temperature, 1.9 °C. Cross-sectional area:  $8.898 \times 10^{-3} \text{ mm}^2$ .

## DISCUSSION

Enhancement of the mechanical performance of muscle by active stretch has been described previously (Fenn, 1924, Hill & Howarth, 1959; Deleze, 1961; Cavagna & Citterio, 1974). The present study, confirming these findings in single fibres, shows that the phenomenon is not due to non-uniformity between fibres in a whole muscle. In addition, the present study elucidates a number of details.

*Force enhancement during stretch*

At all sarcomere lengths studied, the tension was higher than  $P_0$  during stretch (Text-fig. 4A) and depended on the velocity of stretch, i.e. we were studying the well known 'negative' part of the force-velocity relationship (Aubert, 1956; Katz, 1939). This part of the force-velocity relationship has not been delineated in detail in single fibres, but we would expect it to have the form indicated in Text-fig. 3. It is of interest that the force during stretch is often relatively constant after the initial tension rise at the onset of stretch. This behaviour is similar to the constancy of the forces *during shortening* at steady velocities, i.e. similar to the behaviour seen on the 'positive' part of the force-velocity curve. The whole force-velocity relationship is considered to be a characteristic of the contractile machinery. The 'negative' part of this relationship may be further described as showing viscous behaviour of the contractile mechanism during stretch.

The detailed explanation of this viscous behaviour in terms of cross-bridge dynamics is not possible at the present time. We were unable to detect, in the single fibre preparation, a break in the sarcomere lengthening record during stretching, which could be interpreted as cross-bridge deformation (Flitney & Hirst, 1975). This difference in behaviour between the single fibre preparation and whole muscle is likely to be due to greater end compliance in the latter. Introduction of extra compliance in series with a single fibre has indeed been found to result in a break in the sarcomere lengthening record during stretching (F. W. Flitney, personal communication). The 'break' in the force record between the steeply rising part and the flatter part occurred at a stretch of 14–20 nm per half sarcomere, a value similar to that obtained by Flitney & Hirst (1957). However, this occurred at much higher force values in the present study, at a similar velocity of stretch, indicating the considerably greater stiffness of the single fibre preparation. If cross-bridge deformation caused the viscous effect, one would expect the force during stretch to decline when stretch amplitude is increased in such a way as to produce decreased overlap of thick and thin filaments. This does not occur over the range of sarcomere length 2.2–2.8  $\mu\text{m}$  investigated, e.g. Text-fig. 2C. In addition, the magnitude of the force enhancement during stretch is not proportional to overlap (Text-fig. 4A).

This phenomenon has been studied with respect to heat production and ATP splitting. Stretch of actively contracting muscle causes net energy output by the muscle (heat plus work) to go negative (Fenn, 1924; Hill & Howarth, 1959; Abbott, Aubert & Hill, 1951; Abbott & Aubert, 1951). These authors considered the possibility that stretch of actively contracting muscle caused energy to be absorbed into chemical resynthesis. Subsequent measurements of high energy phosphate consump-

tion showed that there was continued chemical breakdown although at a considerably reduced rate (Curtin & Davies, 1972). The biochemical data do not therefore support the view that force enhancement during stretch is based on an increase in activation of the contractile system.

#### *Force enhancement after stretch*

This force enhancement consists of two types of phenomena:

(a) At sarcomere lengths between 1.9 and 2.3  $\mu\text{m}$  there is force enhancement which decays relatively rapidly (in about 1 sec; Text-fig. 6). This force enhancement after stretch in the range of sarcomere length 1.9–2.3  $\mu\text{m}$  is independent of amplitude of stretch greater than approximately 25 nm per sarcomere (Text-fig. 4*B*) and is not associated with a shift of the force–velocity curve (Text-fig. 7).

(b) At sarcomere lengths above about 2.3  $\mu\text{m}$  in addition to a rapidly decaying component, there is force enhancement which decays very slowly (still present after 4.3 sec; Text-fig. 6). Further studies in progress indicate that the effect is still present in 7 sec tetani after the end of tension creep in the control isometric contraction. This would seem to indicate a different mechanism for force enhancement from that for tension creep. The force enhancement after stretch is dependent on amplitude of stretch over the entire range studied (Text-fig. 4*B*) and is associated with a shift of the force–velocity curve to higher force values, i.e. an enhancement of total mechanical performance (Text-fig. 7). The effect increases in magnitude with increase in sarcomere length.

It seems reasonable to consider the first type of force enhancement after stretch as decay of the force acquired due to viscous behaviour during stretch (see above), i.e. some sort of stress relaxation. This would be consistent with the fact that it finally decays completely (Text-fig. 6), and is removed by subsequent shortening of the fibre. The viscous behaviour during stretch was attributed to the contractile machinery (above). One might imagine that the increasing force up to approximately 25 nm per sarcomere resides in the cross-bridges and that the independence of amplitude above that displacement is due to the fact that they ‘give’ and slide. However, after correction for the force enhancement of the second type, the force enhancement for a 20 nm per sarcomere stretch increases with decreasing overlap of thick and thin filaments (Text-fig. 4*B*). It is therefore difficult to explain this behaviour on the basis of current ideas concerning cross-bridge function.

The interpretation of the second type of force enhancement depends to some extent on whether sarcomere uniformity is preserved during and after stretches. The simplest interpretation of the behaviour of the first order diffraction line recorded in these experiments is that dispersion of sarcomere length is not increased by stretch. Gross inhomogeneities such as those which occur during relaxation (Cleworth & Edman, 1972) or in damaged fibres would certainly have been detected, but we cannot rule out the development of some degree of non-uniformity of a random or highly localized nature which cannot be detected with the method used. We shall adopt the simplest interpretation here, that the applied stretches extended all the sarcomeres uniformly, consistent with the findings of Hill (1977), who showed that during active stretch the increase in length occurs in the I band with the A band width remaining relatively constant. Since the extensions produced are greater

than could be allowed by the currently presumed values for the extensibility of the individual elements (Huxley & Simmons, 1971), it seems reasonable to conclude that decreased overlap does occur to some extent, leading to a decreased number of cross projections in apposition to the thick filaments. However, the increase in mechanical performance found is similar to that obtained with a *decrease* in resting sarcomere length over the descending limb of the length-tension curve, i.e. an increase in  $P_0$  with the same  $V_{\max}$  (Edman & Hwang, 1977). The increase in  $P_0$  is usually attributed to the increasing number of cross-bridges in apposition with increased overlap (Gordon *et al.* 1966). Thus in order to explain the effect of active stretch along these lines one has to postulate one or other of the following additional features: (1) that an additional force-bearing mechanism with spring-like properties is recruited in parallel with the shortening elements of the contractile system or (2) that stretch during contraction in some way activates the contractile machinery to produce a higher force than that attained during the ordinary tetanus.

Taking the first of these possibilities, the contractile mechanism behaves here like a shortening unit with parallel viscous (see above) and elastic elements, i.e. a visco-elastic structure. The parallel elastic element analogue for the second type of enhancement takes account of the dependence on amplitude of stretch (Text-fig. 4), the dependence upon sarcomere length (Text-fig. 4), the 'slack length' of the element ( $2.3 \mu\text{m}$ ), and the difference in force-velocity curve converging to a common  $V_{\max}$  (Text-fig. 7). This change in the force-velocity curve would follow from recoil of a parallel elastic element giving an additional force that becomes progressively less with decreasing load due to greater shortening during the load clamp.  $V_{\max}$  would be dependent upon the shortening properties of the active contractile unit alone.

It is difficult to imagine that the structures responsible for the resting tension could act as the element from which the tension enhancement is recruited. We have studied fibres over a range of sarcomere lengths where resting tension is zero. In addition, the stiffness of the 'spring' increases with the sarcomere length at which the fibre is stimulated. Such properties point to the possibility that the structure is formed, reorganized or re-aligned during activation.

In considering the second possibility, i.e. some sort of activation of the contractile machinery by stretch, one encounters difficulty in postulating a mechanism involving changes in cross-bridge function. It seems unlikely that force enhancement after stretch can be explained on the basis of an increase in cross-bridge number in view of the length dependence of the phenomenon, i.e. its increase with decreasing overlap of thick and thin filaments (Text-fig. 4). It is helpful to compare enhancement of mechanical performance by stretch during activity with that produced by other interventions. The phenomenon differs in an important respect from the effects of increased temperature (Hill, 1938; Julian, Steeber & Sollins, 1971) and of hypotonic solution (Edman & Hwang, 1977) both of which produce increases of  $V_{\max}$ . These effects are associated with changes in myosin ATPase activity which seems likely to be the principal determinant of  $V_{\max}$  (Bárány, 1967). It is therefore reasonable to suppose that enhancement of mechanical performance of muscle by stretch during contraction is not due to an enzymic ATPase activation. The possibility exists that stretch during activity alters the function of the cross-bridges to enable them to generate more tension without altering the kinetics which determine  $V_{\max}$ .

Since the force enhancement increases with sarcomere length this mechanism would need to more than compensate for the decreasing overlap of thick and thin filaments.

*Uniformity and stability of sarcomere length in fibres  
stretched during activity*

An important aspect of this phenomenon is that it confers stability to the muscle at long sarcomere lengths. During the rising phase of isometric tension development, sarcomeres which are shorter than average are likely to stretch other sarcomeres in series with them. The stretched sarcomeres now become stronger and resist the pull from the shorter sarcomeres. This has a stabilizing effect in preventing dispersion of sarcomere length. That gross inhomogeneities of sarcomere length do not occur is shown by the small variance of sarcomere length in the isometrically contracting fibre (Text-fig. 8) and the great stability of the width and intensity of the first order diffraction line. It is of considerable interest that this stabilizing mechanism becomes more marked at those sarcomere lengths where its absence would lead to instability, i.e. on the descending limb of the length-tension curve (Text-fig. 4).

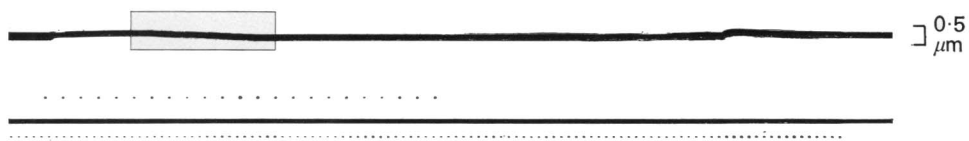
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#### REFERENCES

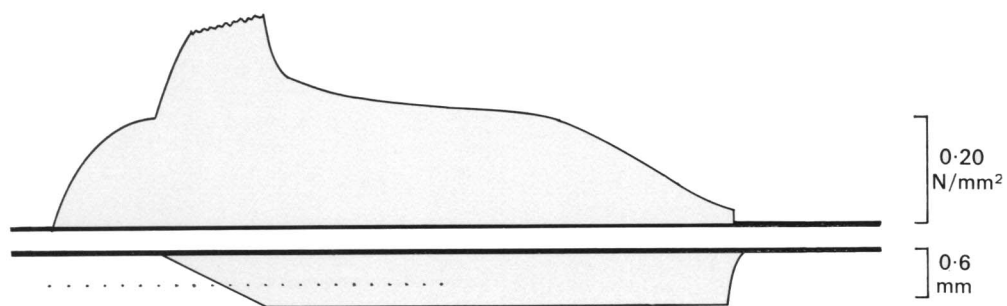
- ABBOTT, B. C. & AUBERT, X. M. (1951). Changes of energy in a muscle during very slow stretches. *Proc. R. Soc. B* **139**, 104-117.
- ABBOTT, B. C., AUBERT, X. M. & HILL, A. V. (1951). The absorption of work by a muscle stretched during a single twitch or a short tetanus. *Proc. R. Soc. B* **139**, 86-104.
- AUBERT, X. (1956). Le couplage energetique de la contraction musculaire. These d'agregation l'enseignement superieur. Editions Arsaia, Bruxelles.
- BÁRÁNY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *J. gen. Physiol.* **50**, 197-218.
- CAVAGNA, G. A. & CITTERIO, G. (1974). Effect of stretching on the elastic characteristics and the contractile component of frog striated muscle. *J. Physiol.* **239**, 1-14.
- CLEWORTH, D. R. & EDMAN, K. A. P. (1972). Changes in sarcomere length during isometric tension development in frog skeletal muscle. *J. Physiol.* **227**, 1-17.
- CURTIN, N. A. & DAVIES, R. E. (1972). Chemical and mechanical changes during stretching of activated frog skeletal muscle. *Cold Spring Harb. Symp. quant. Biol.* **37**, 619-626.
- DELEZE, J. B. (1961). The mechanical properties of the semitendinosus muscle at lengths greater than its length in the body. *J. Physiol.* **158**, 154-164.
- EDMAN, K. A. P. (1966). The relation between length and active tension in isolated semitendinosus fibres of the frog. *J. Physiol.* **183**, 407-417.
- EDMAN, K. A. P. (1975). Mechanical deactivation induced by active shortening in isolated muscle fibres of the frog. *J. Physiol.* **246**, 255-275.
- EDMAN, K. A. P., ELZINGA, G. & NOBLE, M. I. M. (1976). Force enhancement induced by stretch of contracting single isolated muscle fibres of the frog. *J. Physiol.* **258**, 95-96P.
- EDMAN, K. A. P. & HWANG, J. C. (1977). The force-velocity relationship in vertebrate muscle fibres at varied tonicity of the extracellular medium. *J. Physiol.* **269**, 255-272.
- EDMAN, K. A. P. & KIESSLING, A. (1971). The time course of the active state in relation to sarcomere length and movement studied in single skeletal muscle fibres of the frog. *Acta physiol. scand.* **81**, 182-196.
- EDMAN, K. A. P., MULIERI, L. A. & SCUBON-MULIERI, B. (1976). Non-hyperbolic force-velocity relationship in single muscle fibres. *Acta physiol. scand.* **98**, 143-156.
- FENN, W. O. (1924). The relationship between the work performed and the energy liberated in muscular contraction. *J. Physiol.* **58**, 373-395.



A



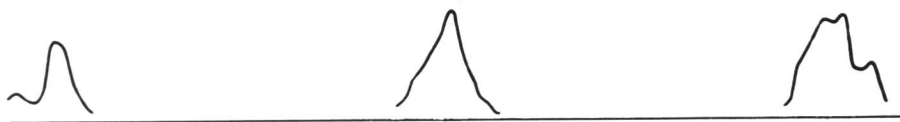
B



C



D



- FLITNEY, F. W. & HIRST, D. G. (1975). Tension responses and sarcomere movements during length changes applied to contracting frog's muscle. *J. Physiol.* **251**, 66-68P.
- GORDON, A. M., HUXLEY, A. F. & JULIAN, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol.* **184**, 170-192.
- HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. B* **126**, 136-195.
- HILL, A. V. & HOWARTH, J. V. (1959). The reversal of chemical reactions in contracting muscle during an applied stretch. *Proc. R. Soc. B* **151**, 169-193.
- HILL, L. (1977). A-band length, striation spacing and tension change on stretch of active muscle. *J. Physiol.* **266**, 667-685.
- HUXLEY, A. F. & SIMMONS, R. M. (1971). Proposed mechanism of force generation in striated muscle. *Nature, Lond.* **233**, 533-538.
- JULIAN, F. J., STEEBER, W. D. & SOLLINS, M. R. (1971). Force-velocity relations of frog twitch muscle fibers obtained during tetani and various times in twitches. *Biophys. J.* **11**, 235a.
- KATZ, B. (1939). The relation between force and speed in muscular contraction. *J. Physiol.* **96**, 54-64.
- MARECHAL, G. (1964). Phosphorylcreatine and ATP changes during shortening and lengthening of stimulated muscle. *Archs int. Physiol. Biochim.* **72**, 306-309.
- RAMSEY, R. W. & STREET, S. F. (1940). The isometric length-tension diagram of isolated skeletal muscle fibres of the frog. *J. cell. comp. Physiol.* **15**, 11-34.
- SNEDECOR, G. W. & COCHRAN, W. G. (1967). *Statistical Methods*. Iowa State University Press.

## EXPLANATION OF PLATE

## PLATE 1

Streak photograph of laser diffraction pattern during stretch of actively contracting fibre. *A*, complete streak photograph showing the zero and first order lines. Stimulus markers are indicated above and time markers (20 msec intervals) below the zero order line. *B*, tension and displacement records and stimulus signals retraced to obtain the same time base as streak record in *A*. *C*, the portion of the first order streak line enclosed by the rectangle in *A* magnified 6×. This section of the streak photograph shows the inward movement of the first order beam as the sarcomeres are stretched, starting from the seventh stimulus marker. *D*, scans of relative intensity against displacement of the first order beam, measured by microdensitometry of the streak photograph at the sixth (before stretch), tenth (during stretch) and fourteenth (after stretch) stimulus markers. Temperature, 1.08 °C. Cross-sectional area:  $19.914 \times 10^{-3} \text{ mm}^2$ .